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X-ray Structure Analysis of a Designed Oligomeric Miniprotein Reveals a Discrete Quaternary Architecture

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Oligomerization, or noncovalent association, is a fundamental strategy for determining protein structural and functional complexity in nature. In this research, miniproteins adopting a defined oligomeric state were designed to study the advantages of protein oligomerization. Peptide 1 is a discretely folded, mixed α/β homo-oligomeric miniprotein motif with only 21 amino acids. The x-ray crystal structure of peptide 1, at 1.2 Å resolution, reveals that it associates as a tetramer (a four-unit oligomer), in which α and β components form a tightly-packed and well-defined hydrophobic core. The miniprotein is thermally stable and shares many of the characteristics of larger proteins.

Miniproteins are short peptide sequences that adopt a discrete protein-like fold in aqueous solution. Therefore, they are valuable minimal models for studying natural proteins. Oligomeric miniproteins, in particular, are good model systems for quaternary structure formation in proteins. Moreover, they are appropriate platforms for determining whether more complex oligomeric structures might ultimately support function.

A homo-oligomeric $\beta\beta\alpha$ miniprotein was derived from a monomeric precursor by a strategy inspired by "domain swapping," an important evolutionary mechanism for protein oligomerization. The monomeric $\beta\beta\alpha$ motif includes intramolecular interactions between α and β secondary structural elements, where α is a helix and $\beta\beta$ is a hairpin. We introduced a bias towards oligomerization by shortening the length of the hinge region between the α and β elements, thereby precluding intermonomer

interactions. A fluorescence-screening assay yielded a homo-oligomeric miniprotein composed of 21 amino acids, with 8 residues in the β hairpin and 12 residues in the α helix. Biophysical studies re-

vealed that this miniprotein forms a defined oligomer in solution and has many of the features of larger proteins, including cooperative folding and a solvent-protected core. However, the exact nature of the miniprotein structure and the forces contributing to the native-protein-like stability remained unknown. We thus set out to determine the crystal structure of peptide **1**, a more stable analog of the original homo-oligomer.

The nature of oligomerization in peptide **1** was revealed by the 1.2 Å x-ray crystal structure, determined using multi-wavelength anomalous diffraction (MAD) phasing. The tetramer consists of four monomers arranged in an antiparallel topology (**Figure 1**), with each monomer flanked by two others oriented in the opposite direction. The tetramer possesses approximate four-fold symmetry: each monomer is slightly closer to one neighbor than the other. The well-defined central hydrophobic core of the tetramer is formed



Authors (top, from left) Dr. Mayssam H. Ali and Prof. Barbara Imperiali, (bottom, from left) Prof. Karen N. Allen and Dr. Ezra Peisach

by residues from one monomer in proximity to core residues from the other three monomers. Those residues on the periphery of the hydrophobic interface interact with residues of the antiparallel monomer nearest them, while the polar residues are solvent-exposed.

Each tetramer is enveloped by a sheath of waters of crystallization with a demarcated water-exclusion zone in the central core region, indicating that no waters can pass into this hydrophobic core. The tet-

rameric structure is highly protein-like in terms of the degree of complexity and the tight packing of side chains. In fact, the relationships between surface area, volume, and molecular weight fit well to a series of empirical relationships for monomeric and oligomeric proteins of varying sizes. The hydrophobic core is defined by five palindromic layers (**Figure 2**) composed of residues from the α -helix and the β -sheet. The packing "register" between monomers might be determined by the conformation-

ally restricted aromatic residues of the core or the interactions made by the hairpin region. The tight packing of the hydrophobic core accounts for the cooperative unfolding and high melting temperatures of peptide **1**. This packing is remarkable in light of the very short linear sequence. Overall, this "miniprotein" structure provides a view of the forces that contribute to oligomerization and a model for understanding quaternary structure in large natural proteins.

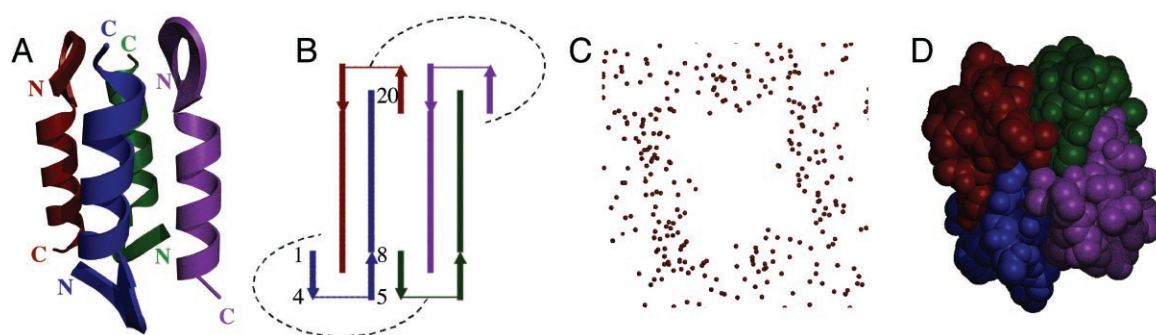


Figure 1. Tetrameric structure of peptide **1**. **A.** Ribbon diagram of tetramer, side view. **B.** Topology diagram. Dashed line indicates inter-monomer interaction. **C.** Crystallographic waters, with void in the shape of the tetramer. **D.** CPK model of peptide **1** indicating space occupied by tetramer. ©1993-2004 by The National Academy of Sciences of the United States of America, all rights reserved.

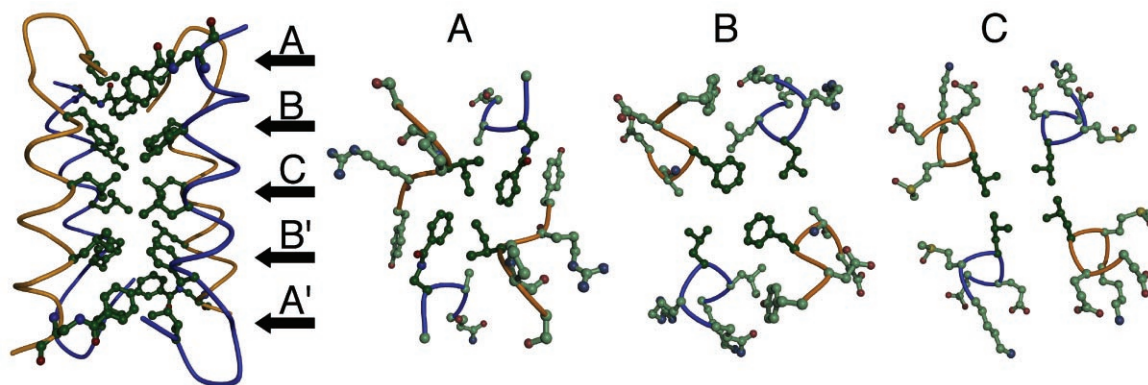


Figure 2. The hydrophobic core of tetrameric peptide **1**. At left, a side view of the tetramer, highlighting the positions of the five palindromic core layers. At right are cross-sectional slices through layers A (Ile-3/DapBz-20), B (Phe-8/Leu-16), and C (Leu-12/Leu-12). Residues comprising the core are in bold. ©1993-2004 by The National Academy of Sciences of the United States of America, all rights reserved.